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# A Review on Analytical Methods for Honey Classification, Identification and Authentication

*Nyuk Ling Chin and Kandhasamy Sowndhararajan*

## Abstract

Authentication of food products is of great concern in the context of food safety and quality. In recent years, interest in honey authenticity in relation with botanical or geographical origin and adulteration has increased. Honey is a ready-to-eat natural food with high nutritional content and gives many health benefits. Authentication of honey has primary importance for both industries and consumers in combatting common honey frauds in the form of mislabeling of honey origin and adulteration with sugar or syrups. Various analytical methods are used for detecting original honey. With a diverse range of equipment and techniques, the conventional analytical methods are still being used in association with advanced techniques as they are part of preliminary screening, processing and product standards. Most of the analytical methods provide indications of pollen distribution, physico-chemical parameters and profile analysis of phenolic, flavonoid, carbohydrate, amino acids, aroma and individual marker components. This review provides an overview and summary of instrumental and analytical methods available for honey authentication from conventional to recent molecular techniques. It is useful as a guide to choosing appropriate method for analysis, classification and authentication of honey.

**Keywords:** adulteration, high fructose sugar syrup, botanical origin, geographical origin, entomological origin

## 1. Introduction

Honey is a natural sweet substance used by human beings since ancient times. The first written evidence of honey was found in a Sumerian tablet dating back to 2100–2000 B.C. [1]. Honey is defined by the European Union as “the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects or the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store, and leave in honeycombs to ripen and mature” [2, 3]. Honey can be classified following its origin, the way it has been harvested and processed. Following honey origin, it is categorised into blossom, honeydew, monofloral and multifloral honeys. Blossom honey is obtained mainly from the nectar of flowers while honeydew or forest honey is

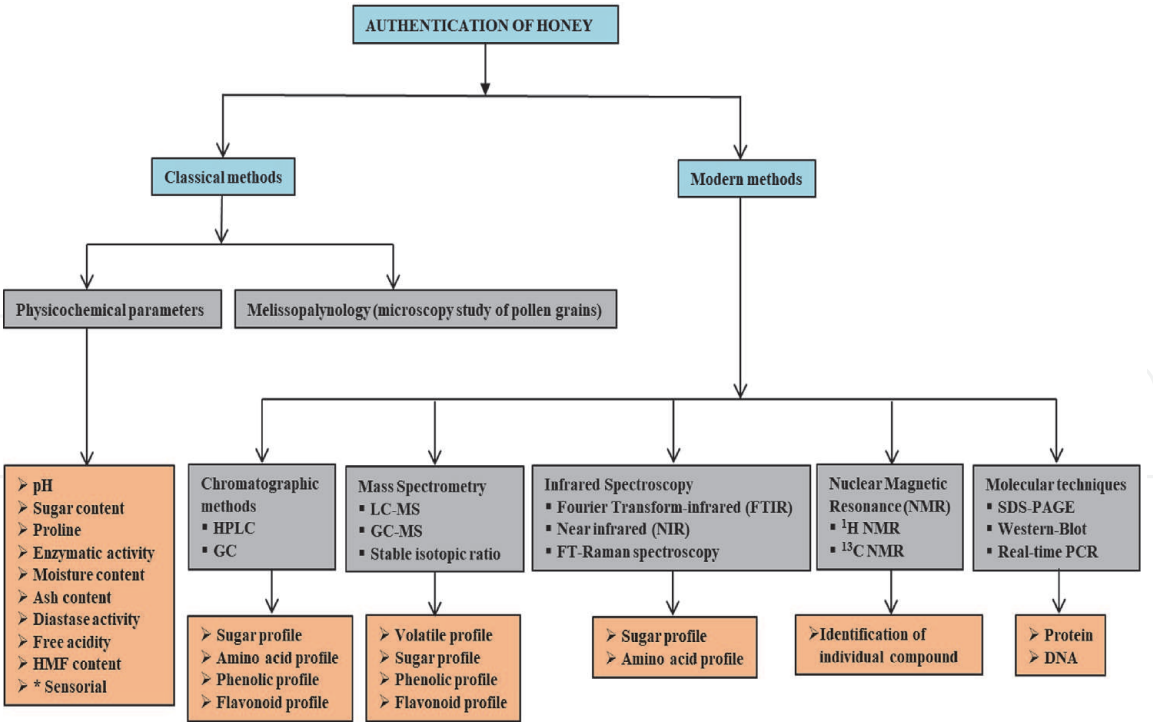
produced by bees after they collect “honeydew” from plant saps. Monofloral honey is arising predominantly from a single botanical origin with above 45% of total pollen content from the same plant species, and is named after that plant, such as citrus, manuka and acacia honey [4]. Multifloral honey is also known as polyfloral honey. It has several botanical sources where none is predominant, for example, meadow blossom honey and forest honey. Honey is an important, distinct and widely used food product for nutrient, cosmetic and medicinal purposes.

The main composition of honey is carbohydrates or sugars, which represent 95% of honey dry weight. Honey is a complex mixture of concentrated sugar solution with main ingredients of fructose and glucose. The average ratio of fructose to glucose is 1.2:1 [5]. Sucrose is present in honey at about 1% of its dry weight. The exact proportion of fructose to glucose in any honey depends largely on the source of the nectar. It also contains bioactive compounds like organic acids, proteins, amino acids, minerals, polyphenols, vitamins and aroma compounds [6, 7]. The protein content of honey is normally less than 0.5% with a small fraction of enzymes. The overall quality of honey such as taste, colour and other physical properties are contributed by the non-volatile compounds like sugar, amino acids, minerals and phenolic compounds while aroma of honey is mainly contributed by the volatile components [8]. The compositional criteria prescribed in existing honey directive are requirements relating to concentrations of acidity, apparent reducing sugar which is calculated as invert sugar and apparent sucrose, 5-hydroxymethylfurfural (HMF), mineral content, moisture and water-insoluble solids [9]. HMF is formed from reducing sugars in honey in acidic environment and often used to evaluate honey quality as it is strongly correlated to ageing and overheating of honey [10]. It is set at maximum limit of 40 mg/kg in honey (with a higher limit of 80 mg/kg for tropical honey) by the Codex Alimentarius Standard commission [11].

Honey has various biological properties including antimicrobial, anti-viral, anti-inflammatory, wound and sunburn healing, antioxidant, anti-parasitic, anti-diabetic, anti-mutagenic and anti-tumoral activities [5, 12–17]. Recent pharmacological studies have revealed that natural honeys have potential to reduce risk of gastric and cardiovascular diseases [18] and have beneficial effects on fertility and ameliorating hormones related to fertility [19–21]. With many beneficial properties, honey is highly priced and is also the major target of adulteration.

## 2. Authenticity of honey

Honey has become the target of adulteration with sugar and/or syrups, for example, cheaper sweeteners from beet or canes like corn syrups (glucose), high fructose corn syrup (HFCS), saccharose syrups, and invert sugar syrups in several countries [22]. In some places, honey is adulterated by bee feeding with sugars or syrups to produce artificial honey. Adulterated honey in the market is often labelled and sold as pure honey and artificial honeys are mislabeled for its botanical or geographical origin [23]. The monofloral honeys are main target for mixing with cheaper multifloral honeys. Monofloral honeys, being the most appreciated by consumers are recognised as better-quality products and they have higher market values [24]. Identification of pure honey and its authenticity have been done based on honey properties. As it becomes an important task for processors, retailers, consumers and regulatory authorities, various analytical methods to measure honey authenticity have been used to detect these honey frauds (**Figure 1**).



**Figure 1.**  
*Conventional and modern analytical methods used for honey authentication.*

**3. Detection methods and technologies**

The classical approach in honey authentication studies is used for determining its botanical origin. Sensory and physicochemical analyses are used in determining monofloral honey origins while the melissopalynological analysis is commonly used to identify floral pollen grains present in honey by microscopic examination [25]. The melissopalynological approach however may not be appropriate for some types of honey like the citrus as the level of pollen content is variable and normally little [26]. The proportion of pollen content is dependent on the plant species, collection season and the nectar yield in male and female flowers. In some cases, pollen can be filtered out in the bee’s honey sac and added fraudulently in honey [27]. Due to the significant natural variation of pollen content, this method is now accompanied with sensory analysis and determination of certain physicochemical characteristics. The number of physicochemical parameters necessary for a complete characterisation is very high and melissopalynology has disadvantages of being slow, very tedious to implement and requires a considerable amount of training.

Due to the limitations of the classical authentication techniques, more reliable modern analytical methods are used to determine botanical and geographical origins of honey. The studies include measuring carbohydrate (sugar) profiles [28], mineral content [29], phenolic and flavonoid compositions [30], aroma profile [31, 32] and amino acid composition [33] using advanced analytical tools like chromatographic techniques [28], mass spectrometry (MS)-based techniques [34, 35], vibrational spectroscopy like infrared (IR) and Raman techniques [36], nuclear magnetic resonance (NMR) [37], stable isotope analysis [38, 39] and others such as flame ionisation detectors (FID) or sensor arrays [40, 41].

Several authors studied on the detection of adulterants like exogenous sugars or additions of sugar syrups by evaluating carbohydrate with different analytical techniques [42–44]. Honey is principally constituted by a mixture of different saccharides such as glucose, fructose, tri- and tetrasaccharides while other



components are present only in very minimum amount [45]. Botanical classification of honey was previously studied using sugar profile and recently high-performance liquid chromatography (HPLC) and chemometric analysis are used to determine sugar profiles of honey [46, 47]. For evaluating floral origin of honey, its volatile composition is determined using headspace solid-phase microextraction (SPME) and gas chromatography coupled to mass spectrometry (GC–MS) [48]. Researchers have also integrated these techniques with chemometric analysis to classify botanical origin of honeys [48, 49]. In chemometric techniques, principal component analysis (PCA) and linear discriminant analysis (LDA) are used to determine the most influencing variables and similarities in studied honey samples [50, 51]. Recent studies have demonstrated the use of molecular genetics approach in determining composition and geographical origins of honey [24], and entomological origins of honey [52–55]. The advantages and limitations of each analytical technique are reviewed and compared in the following sections.

### 3.1 Physicochemical parameters for honey identification

Physicochemical parameters such as pH, sugar content, proline, enzymatic activity, moisture content, ash content, diastase activity, free acidity and hydroxymethylfurfural (HMF) content could provide useful information of honey origin. Nozal Nalda et al. [46] found significant differences of honey samples in terms of 15 mineral contents (except for iron and zinc) and 8 physicochemical parameters (except for sucrose and HMF) in their large-scale study on 73 different honeys of 7 botanical origins of the ling, heather, rosemary, thyme, honeydew, spike lavender and French lavender honeys. The classification of three monofloral Serbian honeys, the acacia, sunflower and linden could be based on variables such as electrical conductivity (0.10–0.76 mS/cm), free acidity (7.80–42.70 meq/kg) and pH (3.17–5.85) [56]. Silvano et al. [57] reported significant differences in the mean value of HMF, colour, electrical conductivity and sucrose content for honeys harvested from different apiaries such as agricultural, hill and meadow zones of the southeast region of Buenos Aires province in Argentina. **Table 1** presents other comparative studies on physicochemical parameters of different types of honeys by investigators from different regions [49, 50, 58–63]. The physiochemical properties of honey are highly dependent on the type of flowers visited by bees, as well as influenced by seasonal, geographical and climatic conditions. Kek et al. [52, 53] have used physicochemical, antioxidant properties and various chemical profiles which included proximate composition, predominant sugars, HMF content, diastase activity, mineral and heavy metal contents to classify honey by its entomological origin, that is, following the bee speciation of honey bees (*Apis spp.*) or stingless bees (*Heterotrigona spp.*).

The sensory properties variation within honeys due to flora in local habitat can be mirrored by pollen analysis or sensorial studies for honey recognition. Stolzenbach et al. [64] reported that Danish honeys had distinct and unique flavours related to its origin of location. Coupled with sensorial analysis, Castro-Vazquez and co-workers used GC–MS to profile volatile compounds of 49 Spanish honey samples from different botanical origins of citrus, rosemary, eucalyptus, lavender, thyme and heather [65] followed by identifying floral marker origins for lavender and lavandin honeys [66]. Lavandin honey is a monofloral product of recent proliferation obtained from a hybrid of the species *Lavandula angustifolia* and *Lavandula latifolia*. In their study, high concentrations of g-nonolactone, farnesol and acetovanillone, which were identified for the first time as components of honey aroma and lactones, dehydrovomifoliol, 4-methoxyacetophenone and decanal were suggested as chemical markers for authenticating lavandin monofloral honey.

Analytical techniques	Samples	References
Melissopalynological	10 Lavender and 10 Lavandin honeys	[66]
Palynological	20 Moroccan honey samples from sunflower, crucifer, carob tree, loeflingia, heather, mint and wood sage	[58]
Sensory properties	205 Slovenian honey from different geographical regions	[50]
	24 multifloral honey samples	[57]
	11 brands of bottled honey from the Indian market	[60]
	21 locally produced Danish honeys	[64]
	49 commercial Spanish monofloral honeys	[65]
	Chesnut honeys	[67]
Physicochemical properties	73 honeys from 7 botanical origins: ling, heather, rosemary, thyme, honeydew, spike lavender and French lavender	[46]* mineral
	30 Uruguayan samples, <i>Eucalyptus</i> spp., <i>Citrus</i> spp., <i>Baccharis</i> spp. and multifloral	[49]* chemical
	205 Slovenian honey from different geographical regions	[50]
	201 samples from 3 unifloral Serbian honeys: acacia, sunflower and linden	[56]
	24 multifloral honey samples	[57]
	20 Moroccan honey samples from sunflower, crucifer, carob tree, loeflingia, heather, mint and wood sage	[58]* colour
	22 Brazilian honey of <i>Eucalyptus</i> and <i>Citrus</i> spp.	[59]* ash
	11 brands of bottled honey from the Indian market	[60]
	26 honey samples from beekeepers in Lithuania	[61]* carbohydrate & electrical conductivity
	77 honey samples: 53 blossom and 24 suspected honeydew	[62]
	67 samples of Indian honeys	[63]* trace metal
*Other properties included in studies besides the general physicochemical properties.		
5 raw Malaysian honey from 4 bee species and 3 commercial honey		[52, 53]* chemical, mineral & antioxidant

**Table 1.**  
*Physicochemical techniques used for identification of honey.*

Similar approach of chemical and sensory characteristics of honey was used to classify chestnut honey geographically [67].

3.2 Chromatographic techniques

Different chromatographic techniques have been reported to determine sugar, amino acids, phenolic and flavonoid profiles of honey samples (Table 2). In the early days, Doner et al. [68] determined maltose/isomaltose ratios of honeys and high fructose corn syrup using gas chromatographic (GC) method. They reported that ratios higher than 0.51 indicated adulteration. Kushnir [69] demonstrated a thin layer chromatography separation for oligosaccharides in honey after sample

Sugar profiles	Samples	References
HPLC	2 nectar and honeydew honeys of different geographical and floral origin	[70]
HPLC-PAD	50 honey samples from different regions of Algeria	[28]
HPAEC-PAD	Fir, rosemary, chestnut and thyme honeys	[43]
	17 artisanal and 8 commercial honeys	[72]
HPLC-DAD	160 honey samples from Acacia, jujube, rape, linden, litchi, clover and multifloral	[73]
HPTLC	15 commercial honeys from 3 types of flowers: lime, polyfloral and acacia	[74]
GC-MS/FID and LC- PAD	280 French honeys from 7 monofloral varieties: 50 acacia, 38 chestnut, 28 rape, 53 lavender, 37 fir, 38 linden and 36 sunflower	[75]
Amino acid profiles		
GC	45 honey samples from the UK, Australia, Argentina and Canada	[76]
HPLC	A variety of different honey samples	[77]
	280 French honeys from 7 monofloral varieties: 50 acacia, 38 chestnut, 28 rape, 53 lavender, 37 fir, 38 linden and 36 sunflower	[78]
	7 different floral types of Serbian honey: acacia, linden, sunflower, rape, basil, giant goldenrod and buckwheat	[80]
LC-ECD	29 honeys: 12 of floral origin and 17 from honeydew	[79]
	10 acacia honeys and 10 rape honeys	[81]
Phenolic and/or flavonoid profiles		
HPLC	9 monofloral eucalyptus honeys from Australia	[82, 84]
	Australian Melaleuca, Guioa, Lophostemon, Banksia and Helianthus honeys	[8, 83]
	Polish honey from heather and buckwheat	[85]
	119 unifloral honeys from 14 different geographical regions	[87]
	7 honey samples: acacia, sulla, thistle and citrus honeys	[88]
	4 types of Spanish honey: floral origin of citrus, rosemary and polyfloral and forest origin of honeydew	[92]
	3 Malaysian tropical honeys: Tualang, Gelam and Borneo and 1 Manuka honey	[93]
HPLC-DAD	40 samples of Robinia honey from Croatia	[30]
	38 sage honey samples from Croatia	[89]
	5 types of honey: 2 milk vetch, 1 wild chrysanthemum, 1 jujube flower and 1 acacia	[94]
HPLC-ECD	Chinese citrus honey	[90]
HPLC-UV	90 Italian honeys	[86]
HPLC-UV and GC-MS	Lemon blossom honey and orange blossom honey	[91]

**Table 2.**  
*Chromatography-based techniques used for honey authentication.*

clean-up by a charcoal-Celite column. Later, Lipp et al. [70] developed a medium-pressure liquid chromatography method using a charcoal/celite mixture for detection of conventional and high fructose syrup in honey at a level as low as 1% of the

total mixture. Honey adulterated with commercial sweeteners could be identified easily based on the sucrose level which was slightly higher than the sucrose content of natural honey [71]. Ouchemoukh et al. [28] investigated sugar profiles of 50 honey samples from different regions of Algeria by HPLC with pulsed amperometric detection (PAD) where 11 sugars were quantified. The average values of fructose and glucose were in the range of 35.99–42.57% and 24.63–35.06%, respectively. The sucrose, maltose, isomaltose, turanose and erlose were found in nearly all the analysed samples, while raffinose and melezitose were present in few samples. Cordella et al. [43] used high performance anion exchange chromatographic method with pulsed amperometric detection (HPAEC-PAD) to detect adulterated honey samples with industrial bee-feeding sugar syrups. Morales et al. [72] determined high molecular weight oligosaccharides of 9 sugar syrups and 25 honey samples using HPAEC-PAD for detection of honey adulterated with corn syrups and high fructose corn syrup (HFCS). Xue et al. [73] developed an HPLC-diode-array detection (DAD) method to detect honey adulteration using rice syrup and identified the adulterant compound from rice syrup as 2-acetylfuran-3-glucopyranoside. Puscas et al. [74] developed a simple and economical analytical method for detecting adulteration of some Romanian honeys based on high-performance thin-layer chromatography (HPTLC) combined with image analysis. The method was then applied for quantitative analysis of glucose, fructose and sucrose contents from different types of commercially available Romanian honeys. Cotte et al. [75] have developed a method using LC coupled with a pulsed amperometric detector (PAD) to assay fructose and glucose, and GC coupled with a flame ionisation detector (FID) to determine the entire profile of di- and trisaccharides.

Gilbert et al. [76] determined 17 free amino acids in 45 honey samples collected from the UK, Australia, Argentina and Canada by using GC. Pawlowska and Armstrong [77] used HPLC methods to determine concentrations of proline, leucine and phenylalanine and their enantiomeric ratios from various honey samples. Cotte et al. [78] claimed to be the first to use HPLC system to discriminate different botanical origins of honey using acid amino analysis. They were successful in characterising Lavender honey. Iglesias et al. [79] developed a reliable and simple method of liquid chromatography–electrochemical detection (LC-ECD) to detect adulteration of acacia honey which was added with rape honey at different levels. Fingerprints of authentic honeys showed that contents of chlorogenic acid were higher in acacia honey, while those of ellagic acid were much lower in rape honey. The free amino acids profile of seven different floral types of Serbian honey (acacia, linden, sunflower, rape, basil, giant goldenrod and buckwheat) from 6 different regions were analysed to discriminate honeys by their botanical origins [80]. Wang et al. [81] suggested chlorogenic acid and ellagic acid as possible markers of acacia and rape honeys authenticity study using LC-ECD.

Currently, research is predominantly focused on determination of phenolic and flavonoid profiles due to their pharmacological properties. Many studies have reported phenolic and flavonoid profiles of honey in relation to botanical and geographical origins using various chromatography techniques [8, 82–87]. The determination of phenolic compounds in honey includes removal of matrix components (especially sugars) and analysis of pre-concentration of analytes using HPLC techniques [88]. Yao et al. [82, 83] analysed phenolic acids and flavonoids along with two abscisic isomers related to botanical origins of nine monofloral Eucalyptus honeys and other five botanical species (*Melaleuca*, *Guioa*, *Lophostemon*, *Banksia* and *Helianthus*) from Australia. In Croatia, flavonoid profiles of Robinia honeys [30] from two production seasons and monofloral sage honey [89] were measured using HPLC/DAD method. The respective honeys showed a common and specific flavonoid profile, but the contents varied between seasons. Liang et al. [90]



established a sensitive and accurate method using HPLC-ECD for simultaneous separation and determination of four phenolic compounds, including caffeic acid, p-coumaric acid, ferulic acid and hesperetin in Chinese citrus honey which were 6–14 times greater than those obtained with DAD. Escriche et al. [91] evaluated flavonoids (naringenin, hesperetin, chrysin, galangin, kaempferol, luteolin, pinocembrin and quercetin) and phenolic acids (caffeic acid and p-coumaric acid) together with 37 volatile compounds in the differentiation between lemon blossom honey (*Citrus limon*) and orange blossom honey (*Citrus spp.*). Naringenin and caffeic acid were the main components in all samples. They have concluded that botanical origin affected the profile of flavonoids and phenolic compounds sufficiently to permit discrimination of honeys, that is, hesperetin in citrus honey, kaempferol, chrysin, pinocembrin, caffeic acid and naringenin in rosemary honey, and myricetin, quercetin, galangin and particularly p-coumaric acid in honeydew honey [92]. Khalil et al. [93] investigated phenolic acid and flavonoid contents of Malaysian Tualang, Gelam and Borneo tropical honeys in comparison with Manuka honey. A total of six phenolic acids (gallic, syringic, benzoic, trans-cinnamic, p-coumaric and caffeic acids) and five flavonoids (catechin, kaempferol, naringenin, luteolin and apigenin) were identified. Jasicka-Misiak et al. [85] determined phenolic profiles of Polish honey samples from heather (*Calluna vulgaris* L.) and buckwheat (*Fagopyrum esculentum* L.). The results revealed that the samples of the same unifloral honeys registered a similar qualitative but slightly quantitatively different phenolic characteristic profiles. Perna et al. [86] identified and quantified phenolic acids, flavonoids and vitamin C in 90 Italian honeys of different botanical origins (chestnut, sulla, eucalyptus, citrus and multifloral) using HPLC–UV analysis. The results showed a similar but quantitatively different phenolic profile of the studied honeys. Zhang et al. [94] described the use of second-order calibration for development of HPLC-DAD method to quantify nine polyphenols in five types of honey samples. Greek unifloral honeys (pine, thyme, fir and orange blossom) were analysed and classified according to botanical origin based on phenolic content (quercetin, myricetin, kaempferol, chrysin and syringic acid) by HPLC analysis [87]. Recently, Campone et al. [88] described a novel approach for a rapid analysis of 5 phenolic acids and 10 flavonoids in honey using dispersive liquid-liquid microextraction followed by HPLC analysis. The proposed new method, compared with commonly used method in analysis of phenolic compounds in honey, provided similar or higher extraction efficiency with exception of the most hydrophilic phenolic acids. These chromatographic techniques provide quite complex chromatograms and coupled with appropriate analyses, can classify honey according to their botanical, geographical and entomological origins.

### 3.3 Mass spectrometry integration with chromatography techniques

The LC–MS and GC–MS methods are used to separate and identify semi-volatile and volatile components in honey. Volatiles influence chiefly to honey flavour and to its variation of floral origin. **Table 3** shows various mass spectrometry techniques used for detection of honey origin. The headspace (HS) solid-phase microextraction (SPME) is the most preferred technique for determining concentration of honey volatiles [91, 95, 96]. With the HS-SPME followed by GC/MC analysis, various volatile components in honey have been detected, identified and quantified, for example, 35 volatile components from Spanish honeys [97], 62 compounds from 28 Greek honeys [98], 31 compounds from 16 samples from mostly European countries [99] and 26 commonly available volatiles in 70 authentic Turkish honey from 9 different floral types [100]. Bianchi et al. [101] developed a HS-SPME method and characterised 40 volatile compounds of Italian thistle honey. In Spain, Soria et al. [102]

Volatile Profiles	Samples	References
GC–MS	80 raw unifloral honey: acacia, sunflower and tilia or lime from Spain, Romania and Czech Republic	[104]
	70 Turkish honey from 15 different geographical regions	[100]*LC/MS for free amino acids
SPME/GC–MS	36 samples of orange honey	[35]*chiral volatile compounds
	42 unifloral honey samples from 5 floral origins: alfafa, sunflower, white clover, carob and calden	[48]*chemometrics
	28 thyme honeys samples from different locations all over Greece	[98]
	Commercial types of European honey	[99]
	46 artisanal honey samples collected in Madrid province, Central Spain	[102]
	40 commercial honey samples	[103]
	Rapeseed, chestnut, orange, acacia, sunflower and linden honeys	[106]*chiral volatile compounds
HS-SPME/GC/MS	7 samples of thistle honey	[101]
	77 unifloral honey samples of different botanical origins	[105] *fingerprinting
HS-SPME/GC × GC-TOF-MS	374 honey samples of Corsican, non-Corsican–French, Italian, Austrian, Irish and German	[31, 34]
SIFT-MS	9 New Zealand honeys: beech honeydew, clover, kamahi, manuka, rata, rewarewa, tawari, thyme and vipers bugloss	[109]
	10 Ohio and Indiana honey samples: star thistle, blueberry, clover, cranberry and wildflower	[110]
SPME and LC-DAD-ESI/MS	Acacia, linden, chestnut, fir, spruce, floral and forest honeys from Slovenia	[51]
HPLC-DAD-MS/MS	Strawberry tree honeys	[111]
	187 honey samples: 98 chaste honey and 89 rape honey	[112]
SPME and UPLC-PDA-MS/MS	Manuka, honeydew, heather, chestnut, and eucalyptus honeys from various geographical origins and ages	[113]
UPLC-Q/TOF-MS	Sunflower, lime, clover, rape and honeydew honey	[114]
Sugar Profile (Carbohydrates and Syrups)		
GC–MS	Honeys from avocado, strawberry tree, willow, loquat, almond tree, fir, evergreen oak, <i>Anthyllis cytisoides</i> , <i>Satureja montana</i> , Teide broom, agave and tajinaste	[45]
	20 honey samples: 16 nectar and 4 honeydew honeys	[107]
	107 floral honeys: heather, rosemary, eucalyptus and citrus	[108]
*Additional feature of measurements.		

**Table 3.**  
*Mass spectrometry-based techniques used for honey authentication.*

differentiated honey origins of mountain and plain by characterising their volatile compositions using 46 artisanal honey samples from different places of Madrid province. Later in 2019, the same co-workers characterised Spanish honeys to their botanical origin using 132 volatile compounds from 40 honey samples [103].

Although volatile compounds can be used to differentiate honey according to country, Juan-Borrás et al. [104] concluded that botanical origin differentiates honey samples better than geographical origin in their research using acacia, sunflower and tilia honeys from Spain, Romania, and Czech Republic. In extension to HS-SPME/GC-MS, Baroni et al. [48] used chemometric to determine organic volatile compound pattern to characterise 42 unifloral honey samples from 5 floral origins while Aliferis et al. [105] used finger-printing to discriminate and classify Greek honeys by their plants and geographical origins. Spanik et al. [106] and Verzera et al. [35] developed a new analytical approach based on enantiomeric ratio investigation of chiral volatile constituents using SPME-GC-MS to evaluate authenticity of honeys.

Besides volatile components in honey, GC-MS has also been used to identify sugar compounds in honey. de la Fuente et al. [45] identified various carbohydrate markers in identifying botanical origins of Spanish honey such as disaccharide maltulose and carbohydrate alcohols of perseitol in avocado honeys and melezitose and quercitol in evergreen oak honeys. Carbohydrate composition of 20 honey samples (16 nectar and 4 honeydew honeys) and 6 syrups has been studied by GC and GC-MS in order to detect differences between both sample groups. The presence of difructose anhydrides (DFAs) in these syrups was described for the first time [107]. Ruiz-Matute et al. [108] developed a GC-MS method for the detection of honey adulteration with high fructose inulin syrups. Inulotriose proved to be the best marker of honey adulteration with these syrups since it was not detected in any of the analysed honey samples.

Beyond HS-SPME, Cajka et al. [31] and Stanimirova et al. [34] investigated the use of the system with comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC  $\times$  GC-TOFMS) to analyse volatile components in honey samples. Langford et al. [109] and Agila and Barringer [110] used selected ion flow tube-mass spectrometry (SIFT-MS), a growing technology that quantifies volatile organic compounds at low concentrations (usually parts-per-trillion, ppt levels) to determine aromas arise from volatile organic compounds in headspace of different monofloral honeys from New Zealand and Ohio, and Indiana, respectively. Furfural, 1-octen-3-ol, butanoic and pentanoic acids were the volatiles with the highest discriminating power among the different types of floral honey [110]. Bertonecelj et al. [51] used diode array detection system and electrospray ionisation mass spectrometry (LC-DAD-ESI/MS) to analyse flavonoid profiles of seven types of Slovenian honey upon solid-phase extraction followed by liquid chromatography to study their botanical origins. The performance liquid chromatography-diode array detection-tandem mass spectrometry (HPLC-DAD-MS/MS) method was used to trace floral origin of strawberry tree honey [111] and chaste honey and rape honeys [112]. Kaempferol, morin and ferulic acid were used as floral markers to distinguish chaste honey from rape honey. Oelschlaegel et al. [113] used photodiode array detection (PDA) with ultra-performance liquid chromatography (UPLC-PDA-MS/MS) to analyse volatile composition of numerous Manuka honeys after solid-phase extraction and identified kojic acid, unedone, 5-methyl-3-furancarboxylic acid, 3-hydroxy-1-(2-methoxyphenyl)penta-1,4-dione and lumichrome in Manuka honey for the first time. Other technologies used include the system of Ultra Performance Liquid Chromatography-Quadrupole/Time of flight-mass spectrometry (UPLC-Q/TOF-MS) where it was possible to identify several components which cannot be detected by diode array using combination of detection with retention time for accurate molecular mass to obtain phenolic acids and flavonoids from ethyl acetate extracts of different honeys (sunflower, lime, clover, rape and honeydew) [114].

3.4 Stable isotopic ratio mass spectrometry (IRMS)

The stable isotope ratio analysis using mass spectrometry may be used to detect adulterated honey samples based on the principle of different  $\delta^{13}\text{C}$  or  $^{13}\text{C}/^{12}\text{C}$  ratio [115–117]. Honey-producing plants, as well as sugar beets belong to the C3 plants while sugar cane, corn and other major source of adulterating syrups are from the C4 plants. Their different photosynthetic pathways result in a different metabolic enrichment of the  $^{13}\text{C}$  isotope. The slower reacting  $^{13}\text{CO}_2$  is depleted to a larger extend in C3 plants than in C4 plants during the  $\text{CO}_2$  fixation (kinetic isotope effect). Thus, it is possible to detect the addition of cheap C4 sugar because of its different  $\delta^{13}\text{C}$  value ranging from  $-22$  to  $-33\text{‰}$  for honey from C3 plants,  $-10$  to  $-20\text{‰}$  for honey from C4 plants and  $-11$  to  $-13.5\text{‰}$  in honey from Crassulacean Acid Metabolism plants (pineapple and cactus). When C4 sugar is added to pure honey, the  $\delta^{13}\text{C}$  value of honey will be altered where honey with  $\delta^{13}\text{C}$  values less negative than  $-23.5\text{‰}$  is suspected to be adulterated. The protein extracted from honey can be used as an internal standard for the determination of adulteration in honey as the corresponding  $\delta^{13}\text{C}$  of protein extract will remain constant. The difference in  $\delta^{13}\text{C}$  between honey and its associated protein extract accepted is  $-18\text{‰}$  deviation at least, which provides the international benchmark of 7% of C4 sugar added [116]. **Table 4** lists honey authentication studies based on IRMS analysis.

Isotope $\delta^{13}\text{C}$	Samples	Reference
IRMS	49 samples of honey	[115]
	40 samples of honey of various botanical origins produced in Brazil, and 8 imported samples, 1 from Argentina, 3 from the USA and 4 from Canada	[116]
	73 Italian honey samples from 6 varieties: chestnut, eucalyptus, heather, sulla, honeydew and wildflower	[117]
	100 pine honey samples	[118]
	13 different brands of honey samples	[120]
	271 Slovenian honey samples from 7 floral types and 4 geographical regions	[38]* $\delta^{15}\text{N}$
EA-IRMS	140 honeys from 7 different botanical origins: acacia, chestnut, rapeseed, lavender, fir, linden and sunflower	[121]
	31 Turkish honey from different sources and regions: flower, pine and chestnut and 43 commercial honey	[123]
	58 honey samples: Northeast China black bee, chaste, acacia, clover, chaste, flowers and jujube honeys	[124]
	Commercial honey	[22]
	516 authentic honeys from 20 European countries	[39]* $\delta^2\text{H}$ , $\delta^{15}\text{N}$ , $\delta^{34}\text{S}$
HPLC-IRMS	79 commercial honey samples	[119]
EA/LC-IRMS	451 authentic honeys	[122]
IRMS and SNIF-NMR	102 French honey from 97 varieties: acacia, chestnut, rape, lavender, fir, linden and multifloral	[44]
Flow isotope IRMS	Manuka honey from New Zealand	[125]

*\*Include other isotopes measurements.*

**Table 4.**  
*Isotope-based techniques to determine botanical and geographical origins of honey.*



Most honey adulteration studies using carbon isotope ratio mass spectrometry (IRMS) are tested on the addition of C4 plant sugars such as HFCS in Turkish pine honey [118], beet sugar products or corn syrup addition in Spanish honey [115] and HFCS, glucose syrup from corn starch and saccharose syrups from beet sugar [22]. Cabanero et al. [119] determined individual sugars like sucrose, glucose and fructose  $^{13}\text{C}$  isotope ratios from honeys of various botanical and geographical origins sources which have been adulterated with beet sugar (C3) and/or C4 sugars like cane sugar, cane syrup, isoglucose syrup and high-fructose corn syrup (HFCS). They developed the first isotopic method that allows beet sugar addition detection. Cengiz et al. [120] provided further validation parameters, such as the limit of detection, limit of quantification and recovery for honey adulteration detection method developed using IRMS.

Besides carbon isotope ratios for sugar and protein, Kropf et al. [38] used the nitrogen stable isotope in honey authentication study using 271 honey samples from 4 different geographical regions of Slovenia while Schellenberg et al. [39] used multiple element stable isotope ratios of hydrogen, carbon, nitrogen and sulphur stable for 516 authentic honeys from 20 European regions. Daniele et al. [121] developed a method to discriminate honeys from seven botanical origins, based on organic acid analysis. The authors suggested that by combining various organic acid contents and values of isotopic ratio through statistical processing by PCA, it is possible to discriminate honey samples as a function of their botanical origin.

The IRMS system used are also enhanced with an elemental analyser and to a liquid chromatograph [122–124] in studies to determine adulteration of C4 plant sugar content in honey. Cotte et al. [44] used site-specific natural isotopic fractionation determined by NMR to first determine their potentials for characterising the substance and then to detect adulteration. They found that the system is limited by detection of a syrup spike starting only at 20%. Frew et al. [125] used a continuous flow IRMS in their adapted method for removing pollen in Manuka honey to improve authenticity detection.

### 3.5 Infrared spectroscopy

Fourier Transform-infrared (FTIR) spectroscopy, near infrared (NIR) and FT-Raman spectroscopy methods have also proved their great potential in food authentication studies. In recent years, these spectroscopic techniques have achieved wide acceptance in the field of food sciences for quantitative and qualitative analysis because of its advantages of collecting high-spectral-resolution data over a wide spectral range. They have been applied successfully in honey authentication studies from the aspects of identification of honey origins and determination of adulterants (**Table 5**).

Ruoff and co-workers have used chemometric evaluations of the spectra measured in various European honeys using near-infrared spectroscopy [126] and front-face fluorescence spectroscopy [127] to authenticate botanical and geographical origins of honey. Important physical and chemical measurands in honey such as sucrose and fructose/glucose ratio using near-infrared spectrometry [128] and by mid-infrared spectrometry (MIR) [129] were also helpful for assessing honey adulteration. Dvash et al. [130] reported the application of NIR reflectance spectroscopy to determine concentration of perseitol, a sugar that is specific to avocado honey. Qiu et al. [131] studied influences of various sample presentation methods and regression models and presented that the spectroscopic technique could accurately determine moisture, fructose, glucose, sucrose and maltose contents in honey samples. Woodcock and co-workers used chemometric tools in NIR spectroscopic studies to differentiate different geographical origins of honey which included Corsican

Technique	Samples	References
NIR	74 commercial honey	[131]
	Avocado honey	[130]
	167 unfiltered honey samples and 125 filtered honey samples	[133]
	Honey from 6 floral origins	[134]
	30 honey samples from Galicia	[135]
	75 honey samples from Ireland	[138]
	68 honey samples from 6 floral origins: <i>Brassica</i> spp., <i>Zizyphus</i> spp., <i>Citrus</i> sp., <i>Robinia pseudoacacia</i> , <i>Vitex negundo</i> var. <i>heterophylla</i> and multiflora	[139]
	144 honey samples: 70 unadulterated honey samples from different apiary of Beijing and 74 honey samples from local grocery stores	[140]*fibre optic diffuse reflectance
	Authentic, unfiltered honeys of Corsican and non-Corsican	[132]* spectral fingerprinting
FTIR	37 honey samples from different regions in the world	[36]
	1075 honey samples from Germany	[136]
FT-NIR	364 samples from 7 unifloral and polyfloral honeys	[126]
	421 honey samples	[128]
FTMIR	144 honey samples from 7 different crops, polyfloral and honeydew honeys	[129]
	Commercial honey, orange blossom, clover and buckwheat	[137]
Front-Face Fluorescence Spectroscopy	371 samples from 10 unifloral and polyfloral honeys	[127]
FT-Raman	Honey samples of clover, orange and buckwheat	[142]
	Corsican samples from other geographical origins: France, Italy, Austria, Germany and Ireland	[143]
i-Raman	74 authentic honey samples from 10 floral origins	[141]
FTMIR-ATR	99 authentic honey samples from artisanal beekeepers in Ireland	[146]
	580 samples of artisanal Irish honeys	[147]
FTIR-ATR	150 honey samples from Europe and South America	[148]
	Honey samples from 4 different states of Mexico	[149]
*Additional features of measurements.		

**Table 5.**  
*Infrared spectroscopy-based techniques used in honey authentication.*

and non-Corsican [132], and honeys from Ireland, Mexico, Spain, Argentina, Czech and Hungary [133]. Using NIR, Liang et al. [134] has successfully classified 147 honey samples from six floral origins at perfect classification rate of 100% and Latorre et al. [135] differentiated Galacian honey by protected “Mel de Galicia” geographical location. Using FTIR, Etzold and Lichtenberg-Kraag [136] determined various botanical origins of honey based on physical–chemical characterisation while Wang et al. [36] determined geographical origins based on sugar profiles. For honey adulterants like commercial syrup including glucose and fructose, Sivakesava

and Irudayaraj [137] classified simple and complex sugar adulterants using mid-NIR, Downey et al. [138] and Zhu et al. [139] have reported the use of transmittance spectra while Chen et al. [140] used a fibre optic diffuse reflectance probe in their NIR systems.

The other different spectroscopy technique in material characterisation is known as the FT-Raman spectroscopy. It measures relative frequencies at which a sample scatters radiation unlike the IR spectroscopy, where it measures absolute frequencies at which a sample absorbs radiation. Li et al. [141] studied the potential of Raman spectroscopy for detecting fructose corn syrup and maltose syrup adulterants in honey. Paradkar and Irudayaraj [142] investigated on adulterants like cane and beet invert in honey. Pierna et al. [143] used FT-Raman spectroscopy to differentiate Corsican honeys from other regions in France, Italy, Austria, Germany and Ireland.

Despite being relevant in honey authentications, IR or Raman spectroscopic techniques are known to give problems to food samples during analysis as prolonged exposure of food sample to laser beam may lead to sample destruction due to heat. The water absorption is very intense in the mid-IR region. A shorter irradiation time and increasing number of scans are recommended to avoid this problem [144]. de la Mata et al. [145] performed attenuated total reflection (ATR) to overcome this problem. FTIR spectroscopy and attenuated total reflection (ATR) sampling technique were used to study botanical origin of honey sample at mid-infrared spectra [146–147]. Hennessy et al. [148] used the similar but with a germanium ATR to verify origin of honey samples from Europe and South America. Gallardo-Velázquez et al. [149] quantified three different adulterants, corn syrup, HFCS and inverted sugar (IS) in honeys of four different locations in Mexico.

### 3.6 Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) spectroscopy provides important structural information for a wide variety of food components and is recognised as one of the main analytical techniques for authentication of food products as it is strongly focused on both structural and chemical characterisation [150, 151]. **Table 6** lists researches which have used NMR technique to authenticate honey samples in relation to geographical or botanical origins and honey adulteration. The more common NMR experiments are the one-dimensional (1D) referred as NMR at  $^1\text{H}$  or  $^{13}\text{C}$  spectra and the two-dimensional (2D) NMR referred as the classical  $^1\text{H}^{13}\text{C}$  heteronuclear multiple-bond correlation (HMBC). One-dimensional  $^1\text{H}$  NMR spectra was used to profile saccharides of honey from different countries [152, 153]. Boffo et al. [154] discriminated botanical origins of Brazilian honeys from the eucalyptus, citrus and wildflower origins. Donarski et al. [37] used cryoprobe  $^1\text{H}$  NMR spectroscopy for verification of Corsican honey's geographical locations in Europe and later used biomarkers to identify botanical origins of sweet chestnut and strawberry-tree honeys [155]. Zielinski et al. [3] proposed phenylacetic acid and dehydrovomifoliol as markers of Polish heather honey, confirmed 4-(1-hydroxy-1-methylethyl)cyclohexane-1,3-dienecarboxylic acid as a marker of lime honey and reported that formic acid and tyrosine as the most common characteristic compounds of buckwheat honey. Using signals of protons and carbon of the methylene group of quercitol in  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of honey, Simova et al. [156] identified and discriminated oak honeydew honey from all other honey types of honeydew honeys. Quercitol is considered as a good botanical marker for the genus *Quercus* which the oak tree belongs. Beretta et al. [157] used  $^1\text{H}$  NMR profile coupled with electrospray ionisation-mass spectrometry (ESI-MS) and two-dimensional NMR analyses to seek reliable markers of the botanical origin of Italian

Spectrum	Samples	References
<sup>1</sup> H NMR	35 honey samples from multifloral, heather, lime, rape, buckwheat and acacia	[3]
	180 Corsican honey samples	[37]
	57 samples from different countries	[153]
	46 honey samples from flowers of citrus, eucalyptus, assa-peixe and wildflowers	[154]
	374 samples from Austria, France, Germany, Ireland and Italy	[155]
	118 honey samples of 4 different botanical origins: chestnut, acacia, linden and polyfloral	[159] *chloroform extracts
	353 honey samples from acacia, chestnut, linden, orange, eucalyptus, honeydew and polyfloral	[160] *chloroform extracts
<sup>1</sup> H NMR and <sup>13</sup> C NMR	23 samples of polyfloral and 18 samples of acacia honey	[152]
	24 honey samples of oak and others	[156]
<sup>1</sup> H NMR and <sup>1</sup> H- <sup>13</sup> C HMBC	63 authentic and 63 adulterated honey samples	[42]
	71 honey samples: robinia, chestnut, citrus, eucalyptus and polyfloral	[158]
<sup>1</sup> H NMR-ESI-MS and <sup>1</sup> H- <sup>13</sup> C HMBC	44 commercial Italian honeys from 20 different botanical sources	[157]
LF <sup>1</sup> H NMR	Pure blossom honey samples	[162]
	80 samples from eucalyptus, orange, Barbados cherry, cashew tree, assa-peixe, assa-lipto and Cipo-Uva	[161]

*\*Additional features of measurements.*

**Table 6.**  
*Nuclear magnetic resonance (NMR) techniques in honey authentication.*

honeys. Lolli et al. [158] used both <sup>1</sup>H NMR and heteronuclear multiple bond correlation spectroscopy (HMBC) to characterise five different floral sources of Italian honey. <sup>1</sup>H NMR spectra of chloroform extracts was developed and used to study non-volatile organic honey components for botanical origin characterisation of chestnut, acacia, linden and polyfloral honeys where specific markers were identified for each of the six monofloral Italian honeys [159, 160]. Bertelli et al. [42] investigated adulterated honey falsified by intentional addition of different concentrations of commercial sugar syrups using one-dimensional (1D) and two-dimensional (2D) NMR coupled with multivariate statistical analysis. Ribeiro and co-workers used low field nuclear magnetic resonance spectroscopy (LF <sup>1</sup>H NMR) to classify Brazilian honeys into eight different botanical sources [161] and to differentiate honey adulterated by HFCS in different concentrations from 0% (pure honey) to 100% (pure high fructose corn syrup) [162].

3.7 Molecular techniques

Honey contains only about 0.2% protein [163] and it originates from bee and nectar of plants [164–165]. Honey proteins appear in the form of enzymes, predominantly diastase (amylase), invertase and glucose oxidase. Others, including catalase and acid phosphatase, can also be present, depending on the type of floral source and recently proteolytic enzymes have been described in honey. The major



proteins in honey possess different molecular weights depending on its bee species. Thus, protein- and DNA-based honey authentication methods such as the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and real-time PCR are used to identify, authenticate and classify honey samples (Table 7).

Marshall and Williams [166] used SDS-PAGE, the high-resolution two-dimensional electrophoresis method with methylamine-incorporating silver stain to characterise trace proteins of Australian honeys. Their study revealed that honey protein constituents are predominantly of bee origin. Lee et al. [167] used

Protein-based methods	Samples	References
Western-blot	Honeys from <i>Prosopis caldenia</i> , <i>Prosopis</i> sp., <i>Eucalyptus</i> sp., <i>Helianthus annuus</i> , <i>Melilotus albus</i> and <i>Larrea divaricata</i>	[168]
	Honey samples from beekeepers and markets throughout Korea	[169]
	RJ proteins from Slovakia	[170]
ELISA	RJ protein Apalbumin 1 in honey samples from acacia, linden, rapeseed, dandelion and chestnut	[171]
SDS-PAGE	Native and foreign bee-honeys	[167]
	Honeys from <i>Prosopis caldenia</i> , <i>Prosopis</i> sp., <i>Eucalyptus</i> sp., <i>Helianthus annuus</i> , <i>Melilotus albus</i> and <i>Larrea divaricata</i>	[168]
	Honey samples from beekeepers and markets throughout Korea	[169]
	Australian honey samples	[166]* high-resolution 2-dimensional electrophoresis
DNA-based methods		
Manual tracking plant, fungal, and bacterial DNA	Commercial, eucalyptus and lemon honeys	[174]
Manual, QIAQuick PCR Purification Kit	Honeydew honey with multifloral, wild flower and rape honeys, <i>Acacia</i> -with multifloral honey	[177]
CTAB	1 pine honey, 2 wild honey, 5 polyfloral honey	[175]* pollen
	3 different apiaries	[176]
Other commercial kits methods besides CTAB		
NucleoSpin Plant, Wizard methods and DNeasy Plant Mini Kit	<i>Calluna vulgaris</i> , <i>Lavandula</i> spp., <i>Eucalyptus</i> spp. and a multifloral honey	[24]
NucleoSpin Isolation Food Kit, Wizard Magnetic DNA Purification and DNeasy Mericon Food Kit	14 types of raw honey from <i>Apis dorsata</i> , <i>Apis mellifera</i> , <i>Apis cerana</i> and <i>Heterotrigona itama</i>	[54, 55]
DNeasy Tissue Kit	One regional origin (Pyrenean honey) and one worldwide mix of different honeys (wild flower honey)	[172]
RJ, Royal jelly. *Additional features of measurements.		

Table 7.  
Molecular techniques for honey authentication.

SDS-PAGE to differentiate native bee-honey (NBH) and foreign bee-honey (FBH) from different molecular weight found in major protein of NBH at 56 kDa and FBH at 59 kDa which were used as protein markers to differentiate NBH and FBH. Baroni et al. [168] reported on the development of a novel method based on honey proteins to determine floral origin of honey samples using SDS-PAGE immunoblot or Western-Blot techniques. The Western-Blot is done to confirm the presence, absence and expression level of a protein of interest using specific antibodies while SDS-PAGE separates protein based on molecular weight. Won et al. [169] distinguished honey produced by two different bee species, *Apis mellifera* and *Apis cerana* by the difference in molecular weight of their major proteins (56 and 59 kDa) using SDS-PAGE and later used the purified proteins as antigens for antibody reactions in rats. The Western-blot method verified differences in major proteins' surface structure thus can be used to differentiate the different honey bee species. Besides honey, royal jelly, another product secreted by honeybee workers as food to be fed to the larvae which will be raised as the potential queen bee, is known to have high protein contents. Simuth et al. [170] reported the presence of royal jelly (RJ) proteins in honey collected from nectars of different plants, origin and regions and in honeybee's pollen by Western-blot analysis using polyclonal antibodies raised against water-soluble RJ-proteins. They authors suggested that the Apalbumin-1 is a common component of honeybee products and thus is an appropriate marker tool for detecting adulteration of honey by means of immunochemical methods. Bilikova and Simuth [171] developed the 55 kDa major protein of royal jelly, named apalbumin 1 (an authentic protein of honey and pollen pellet), and quantified it by an enzyme-linked immunosorbent assay (ELISA) using specific polyclonal anti-apalbumin 1 antibody.

In recent food authentication methods, DNA-based method is used and regarded as the most reliable, rapid and reproducible technique to detect adulteration and origin of food materials (Table 7). Valentini et al. [172] proposed a new method for investigating plant diversity and geographical origin of honey using a DNA barcoding approach that combines universal primers and massive parallel pyrosequencing. Laube et al. [173] developed a DNA-based method for characterisation of plant species in honey which further used as geographical origin indication. They identified PCR markers for detection of plant species related to "Miel de Corse", a protected designation of origin honey and "Miel de Galicia", a protected geographical indication (PGI) honey as well as German and English honeys. Soares et al. [24] used DNA-based methods to identify botanical species of honey. They used five DNA extraction methods combined with three different sample pre-treatments on four honey samples, three monofloral and one multifloral. The different DNA extraction procedures were compared in terms of DNA integrity, yield, purity and amplification targeting universal and ADH1 specific genes of *C. vulgaris* where they identified monofloral heather honey successfully. Although these molecular techniques give appreciable results, a prior knowledge about the plant species is required to identify origin of honey samples. Besides detecting plants, Olivieri et al. [174] used DNA-based methods to detect fungi and bacteria in honey due to potential risks evoked by microorganisms, allergens or genetically modified organisms. Guertler et al. [175] developed an automated DNA extraction method from pollen in honey. The authors altered several components and extraction parameters and compared the optimised method with a manual CTAB buffer-based DNA isolation method. The automated DNA extraction was faster and resulted in higher DNA yield and sufficient DNA purity. The results obtained from real-time PCR after automated DNA extraction are also comparable to that of manual DNA extraction procedure. Jain et al. [176] introduced a protocol for DNA extraction from honey using modified CTAB-based protocol. Waiblinger et al. [177] described an in-house

and interlaboratory validation of a DNA extraction method from pollen in a unifloral rape honey with several multifloral honeys. While most DNA studies on honey have focused on botanical species identification, Kek et al. [54, 55] have determined the best DNA extraction of bees in honey and introduced entomological identification of honey based on bee mitochondrial 16S rRNA and COI gene sequences.

3.8 Other techniques

**Table 8** presents other methods which included instrumental and improvised analytical procedures used for honey identification and authentication. Cordella et al. [178–179] used differential scanning calorimetry (DSC) to study thermal behaviour of honeys to detect adulterations effects, that is, sugar syrups and classify honeys (Robinia, Lavender, Chestnut and Fir). Hernandez et al. [180] characterised different types of honey produced in the Canary Islands according to their mineral contents using atomic absorption spectrophotometry. Guo et al. [181] used an open-ended coaxial-line technology and a network analyser at 10–4500 MH to detect sucrose–adulterated honey using a sucrose content sensor where permittivity of different adulterated and pure honeys was measured. Pure honey possessed higher dielectric constant when compared with pure sucrose syrup. Roshan et al. [182] used UV spectroscopy together with chemometric techniques to develop a simple model developed to describe authentication of monofloral Yemeni Sidr honey. Tuberoso et al. [183] assessed colour coordinates of 17 unifloral honeys types from different geographic locations in Europe using spectrophotometric method. Wang et al. [184] determined geographical origin of honey based on fingerprinting and

Techniques	Samples	References
Gravimetric method for ash	22 eucalyptus and citrus honey samples from Brazil	[59]
Differential scanning calorimetry	<i>Lavandula</i> , <i>Robinia</i> and <i>Fir</i> honeys	[179]
Atomic absorption spectrophotometry	116 samples of monofloral and multifloral honeys	[180]
Dielectric properties of honey	Chinese jujube, yellow locust tree and Chinese milk-vetch	[181]
UV spectroscopy	38 honey (13 genuine monofloral Sidr honeys ( <i>Ziziphus spinachristi</i> ), 14 Sidr honeys, 5 polyfloral and 6 non-Sidr honeys)	[182]
UV–visible spectrophotometer	305 samples from 17 unifloral honey types	[183]
Fingerprinting and barcoding of proteins by MALDI TOF MS	16 honey samples	[184]
Digital image-based flow-batch system	210 honey samples from southwest of the province of Buenos Aires and Argentina	[185]
Multivariate analysis of colour and mineral composition	77 honey samples collected in Spain	[186]
Atomic absorption spectrometry	6 monofloral honeys and 2 multifloral Spanish honeys	[187]
ICP-MS	163 honey samples from 4 types of honey: linden, vitex, rape and acacia	[188]

**Table 8.**  
*Other techniques used in honey authentication.*

barcoding of proteins in honey by using matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI TOF MS). The protein fingerprints were used to differentiate geographical origins of honey samples produced from different countries and various states of the USA, including Hawaii. Dominguez et al. [185] studied geographic origin classification of honey samples from Argentina by a conventional flow-batch system with a simple webcam to capture digital images. In this technique, analytical information is generated from colour histograms obtained from digital images using different colour models such as red–green–blue (RGB), hue–saturation–brightness (HSB) and Grayscale. Gonzalez-Miret et al. [186] analysed mineral content and colour characteristics of 77 honey samples to classify them following botanical origin. de Alda-Garcilope et al. [187] characterised six monofloral honeys and two multifloral Spanish honeys to their protected designation of origin “Miel de Granada” using their metal content. Chen et al. [188] used inductively coupled plasma mass spectrometry (ICP-MS) and chemometrics data of 12 mineral elements to classify Chinese honeys to their botanical origins. Using simple gravimetric method, Felsner et al. [59] characterised monofloral honeys by its ash content, a parameter that has been associated with floral source of honey samples with the hierarchical design. For analysis of honey classification for authentication purposes, data collected needs strong statistical analysis such as multivariate analysis, regression analysis or chemometrics like principal component analysis (PCA) and linear discriminant analysis (LDA). More advanced techniques include the chemical finger printing technique for indicating a unique pattern.

#### **4. Conclusion**

Determination of honey authenticity by its geographical or botanical origins and its purity is the most important criteria to ensure its safety and quality. The older techniques of melissopalimnology, for example, may need to be coupled with newer and more advanced technique to provide higher precision and accuracy of investigation. Strong analytical methods and procedures are necessary for in-depth analysis of data obtained from instrumental measurements for meaningful research in honey authentication. With new knowledge and information of honey origins and authenticity, there rise the need to update current standards of the Codex Alimentarius and the European Union to incorporate newer information and guidelines for standardisation of honey qualities with respect to authenticity. Newer profiles may include components like the aliphatic organic acids, amino acids, volatile components, flavonoids, carbohydrates, phenolic acids and proteins instead of just the sugar profiles. Guidelines towards discrimination botanical and geographical origin may also be implemented. This review provides insights to encourage researchers to further explore novel detection technologies in authentication studies of food materials.



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### **Author details**

Nyuk Ling Chin<sup>1\*</sup> and Kandhasamy Sowndhararajan<sup>1,2</sup>

1 Department of Process and Food Engineering, Faculty of Engineering,  
Universiti ePutra Malaysia, Serdang, Selangor, Malaysia

2 Department of Botany, Kongunadu Arts and Science College, Coimbatore,  
Tamil Nadu, India

\*Address all correspondence to: chinnl@upm.edu.my

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